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Synthetic, Spectroscopic And Antibacterial Studies Of Fe(II),Co(II),Ni(II),Cu(II),Zn(II),Cd(II)and Hg (II),Mixed Ligand Complexes Of Saccharin And Amoxicillin(antibiotics)

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Abstract

Mixed ligand complexes of bivalent metal ions, viz; M= Fe(II),Co(II),Ni(II),Cu(II), Zn(II), Cd (II), and Hg(II) of the composition Na2[M (Amox)(Sac)3] in 1:1:3 molar ratio, (where Amox = Amoxicillin tryhydrate (C16H19N3O5S.H2O) and Sac = Saccharine(C7H5NO3S) have been synthesized and characterized by repeated melting point determination, Solubility, Molar conductivity, determination the percentage of the metal in the complexes by flame(AAS), FT-IR, magnetic susceptibility measurements and electronic spectral data. The ligands and their metal complexes have been screened for their biological activity against selected microbial strains (gram +ve) and (gram -ve).

Key words:(Amoxicillin antibiotics, Saccharine, mixed ligand) Complexes, Antibacterial activities, and spectral studies

1. Introduction

The study of mixed ligand complex formation is relevant in the field of analytical chemistry, where the use of mixed ligand complexes allows the development of methods with increased selectivity and sensitivity. They have also great importance in the field of biological and environmental chemistry [1] .Amoxicillin tryhydrate ($C_{16}H_{19}N_3O_5S.H_2O$) is chemically *(2S.5R.6R)*-6-{[(*2R*)-2-amino-2-(4-hydrowyphenyl)-acetyl]amino}-3,3-dimethyl-7-oxo-4-thia-l-azabicyclo [*3.2.0*] heptane-*2*-carboxylic acid acts by inhibiting the synthesis of bacterial cell wall. [2] Juan Anacona and Ibranyellis [3].Synthesized cephalexin(ceph) with transition and d^{10} metal ions have been investigated. The complexes [M(ceph)Cl].nH_2O [M= Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Hg(II)] were characterized by physicochemical and spectroscopic methods. The IR and 1H NMR spectra of the complexes suggest that cephalexin behaves as a monoanionic tridentate ligand. In vitro antibacterial activities of cephalexin and the complexes were tested.[4]

Saccharin (C7H5NO3S), also called o-sulfobenzoimide, is widely used as an artificial sweetening agent. Saccharin is a weak acid [5] .Studies of mixed ligand complexes containing saccharinate and some other ligands have demonstrated that the coordination mode of saccharinate is highly adaptable to the steric requirements of the complex formed. [6-7].The saccharinato salts and complexes are thus, both structurally and spectroscopically, well investigated. Recently, however, the research work in this field has been expanded over the adducts of the saccharinates with various aromatic nitrogen bases. [8-9].

This paper describer the synthesis and characterization of several (transition and d10) metal ions mixed ligand Complexes Of Saccharine and Amoxicillin(antibiotics) in 1:3:1(M: Sac: Amo) ratios and biological evaluation (in vitro antibacterial activities) of the complexes were tested.The behavior of two ligands toward M (II) ion was studied. The characterization of the prepared compounds was performed using different physicochemical methods.

2. Experimental

2.1. Chemicals

All chemicals used were of reagent grade and were used as received.$FeCl_2.9H_2O$, $CoCl_2.6H_2O$,$NiCl_2.6H_2O$, $CuCl_2.2H_2O$, $CdCl_2.H_2O$,$HgCl_2$, $ZnCl_2$, Na OH (supplied by either Merck or Fluka) ethanol, methanol dimethylforamaide, dimethyl sulfoxide and KBr, from (B.D.H). Amoxicillin powder DSM (Spain) and Saccharine from Riedial- Dehaen.

2.2. Instrumentals

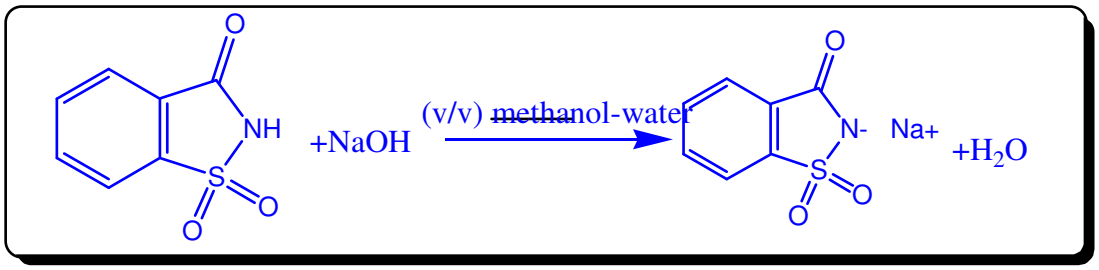
UV-Vis spectra were recorded on a (Shimadzu UV- 160A) Ultra Violet-Visible Spectrophotometer. IR-spectra were taken on a (Shimadzu, FTI R- 8400S) Fourier Transform Infrared Spectrophotometer (4000-400) cm^{-1} with samples prepared as KBr discs. Metal contents of the complexes were determined by atomic absorption(A.A)technique using a Shimadzu AA 620G atomic absorption spectrophotometer. The Chloride contents of complexes were determined by potentiometric titration method using (686-Titro processor-665. Dosimat Metrohn Swiss). Conductivities were measured for 10^{-3}M of complexes in DMSO at 25°C using (conductivity meter, Jewnwary, model 4070). Magnetic measurements were recorded on a Bruker BM6

instrument at 298°K following the Farady's method .. In addition melting points were obtained using (Stuart Melting Point Apparatus). The proposed molecular structure of the complexes were drawing by using chem. office prog, 3DX (2006).

2.3 Synthesis of (Mixing ligands) complexes with some metal ions

(2.3.1) Synthesis of Na_2 [Cu(Amox)$(Sac)_3$] complex:

sodium saccharinate $C_7H_4NO_3SNa$ [0.549,gm(3mmol)]of saccharin with [0.12 gm (3mmol)] solution of sodium hydroxide in (v/v) methanol-water was deprotonated according to the following reaction [8] scheme (1)



Scheme (1): preparation of Sodium saccharinate (Saccharin - sodium salt)

(2.3.2) A solution of (Amoxicillin) (0.365 gm, m mole) in methanol (5 ml) with NaOH (0.04 gm, m mole) and a solution of sodium saccharinate solution (0. 612g, 3mmol) were added to stirred of Cu(II) Chloride dihydrate (0.17 gm, mmole) in methanol (5 ml). The resulting mixture was stirred for (2 hours). Then the mixture was filtered and washed with an excess of ethanol and dried at room temperature during (24 hours). A green solid was obtained, m.p (188º C) ,yield 86% .

(2.3.3) Synthesis of Na_2 [Fe(Amox)$(Sac)_3$], Na_2 [Co(Amox)$(Sac)_3$] , Na_2 [Ni(Amox)$(Sac)_3$] , Na_2 [Zn(Amox)$(Sac)_3$] .Na_2 [Cd(Amox)$(Sac)_3$] and Na_2 [Hg (Amox)$(Sac)_3$] complexes : scheme (2)

The method used to prepare these complexes was similar method to that mentioned in preparation of Na_2 [Cu(Amox)$(Sac)_3$] complex in paragraph (2.3.2) with good yields(85-89) % .

3. Results and Discussion

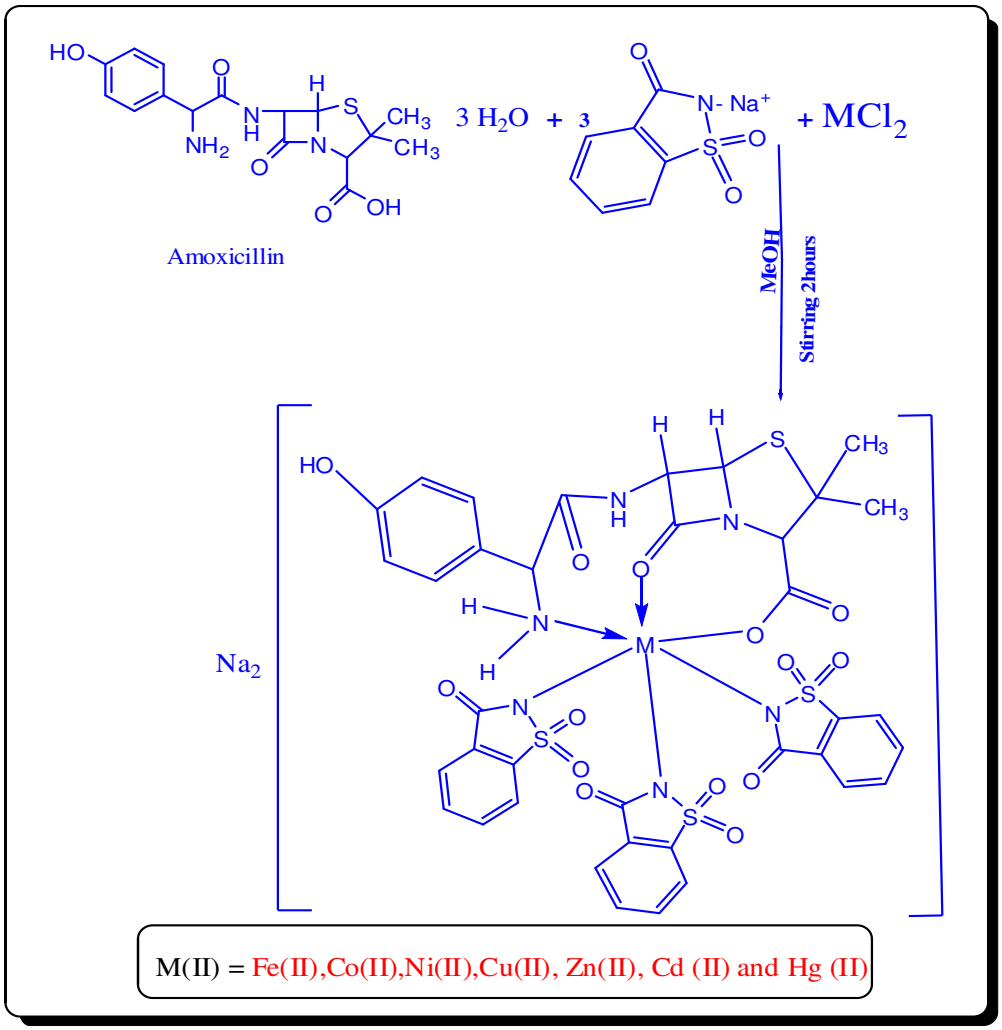
3.1. Characterization of Metal Complexes.

Generally, the complexes were prepared by reacting the respective metal salts with the ligands using 1:1:3 mole ratios, .[M: Amox:3(Sac)], i.e. one mole of metal salt : one mole of amoxicillin and three moles of sodium Saccharinate. The synthesis of mixed ligand Metal complexes may be represented as follows see Scheme *(2):*

$3Sac\ H + 2NaOH \rightarrow 3Sac^- Na^+ + 3H_2O$

$3\ Sac^- Na^+ + Amox + MCl_2 \rightarrow Na_2[M(Sac)_3(Amox)] + 4H_2O + NaCl$

(where Amox is Amoxicillin and Sac H is Saccharin).



Scheme (2): Schematic representation Preparation of the Complexes $Na_2[M(Amox)(Sac)_3]$

The formula weights and melting points, are given in(Table I).Based on the physicochemical characteristics, it was found that all the complexes were non-hygroscopic, stable at room temperature and appears as powders with high melting points. The solubility of the complexes of ligands was studied in various solvents .They are not soluble in water .All complexes are soluble in dimethyl formamide; (DMF)and dimethylsulfoxide (DMSO) solvent. The complexes were analyzed for their metal by atomic absorption measurements and chloride contents were determined by standard methods [10].(Table-1) for all complexes gave approximated values for theoretical values. The observed molar conductance values measured in DMSO (10^{-3}M solution) at room temperature lie in the (54.76- 73.18) Ω^{-1} cm^2 mol^{-1} range, indicating their electrolytic nature with(1: 2). [11].
The atomic absorption measurements and Chloride contents (Table-1) for all complexes gave approximated values for theoretical values.

3.2. FT-IR of $Na_2[Fe(Amox)(Sac)_3]$ (1), $Na_2[Co(Amox)(Sac)_3]$ (2), $Na_2[Ni(Amox)(Sac)_3]$(3), $Na_2[Cu(Amox)(Sac)_3]$ (4) , $Na_2[Zn(Amox)(Sac)_3]$ (5)$Na_2[Hg(Amox)(Sac)_3]$ (6) and $Na_2[Cd(Amox)(Sac)_3]$ (7) complexes:

The relevant vibration bands of the free ligands and there complexes were recorded in KBr in the region 4000–400 cm^{-1}.The assignment of the characteristic bands (FT-IR) spectra for the free ligand(Amox), figure(1) and (SacH), Figure(2) are summarized in Table (2) and (3)respectively .The characteristic frequencies of the (1),(2),(3),(4), (5),(6) and (7) metal complexes are given in(Table-4) .Interpretation of IR bands of the complex have been carried out comparing with the spectrum of IR of amoxicillin, Saccharin and related compound have been well studied [13-21]
.Generally, free primary amino groups exhibit two N–H stretching frequencies in the

3300 to 3500 cm^{-1} region which are shifted by about 100 to 200 cm^{-1} to lower wave number after coordination with a metal ion. [15]Since the prepared compounds contain both primary NH_2 and secondary NH groups, peaks corresponding to these groups appear in the IR spectra.
The FT-IR spectrum for the starting materials (Amox) and (SacH) , which exhibits band at (3456), and (3367, 3159) cm^{-1} due to υ(N–H) primary amine stretching vibration, on complexation this band has been shifted to lower frequencies (3344), (3387), (3332) , (3444), (3463) , (3444) and (3463) $cm^{-1}$for complexes (1), (2), (3), (4), (5), (6) and (7), showing that the coordination is through the nitrogen atom of amine group. The band at (1685) cm^{-1} stretching vibration is due to υ (C=O) for β-Lactam group, these band has been shifted to lower frequency (1647), (1643), (1741),(1728),(1643) ,and(1639) cm^{-1} for complexes (1),(2),(3),(4), (5),(6),and (7),showing that the coordination is through the Oxygen atom of β-Lactam group. The bands at (1585), and (1396) cm^{-1} were assigned to stretching vibration (COO-) asymmetric and symmetric stretching vibration, respectively, on complexation these bands have been shifted to lower and higher frequencies [(1595.), (1585), (1589), (1631), (1612), (1589), and (1585) cm^{-1} for υ as (COO^{-})], and [(1374), (1369), (1369), (1369), and (1369) cm^{-1},for υs(COO–)] for the compounds (1), (2), (3), (4), (5), (6),and (7) respectively, This shift agrees with earlier reports and is an indication of chelation of the ligand through the carboxylate groups to the metal ions and that the coordination with metal was occurred through the oxygen atom of carboxylate ion. [16-18] Moreover, Δ(a υs (COO^{-}) - υs(COO^{-}) values of complexes below 200 cm^{-1} would be expected for bridging or chelating carboxylates but greater than 200 cm^{-1} for the monodentate bonding carboxylate anions [17].The bands in the ligand (Sac) due to νas (SO_2) and νs(SO_2) appear at 1292 cm^{-1} and 1149 cm^{-1} respectively. These bands almost remain unchanged in the complexes indicating that this –SO_2 group is not participating in coordination.[1 9]As it was shown [20], the stretching vibrations of the sulphonyl group can be considered as good group vibrations. The presence of other bands originating from the ligand internal modes in the same region in the spectra of the saccharinates, however, can often complicate the assignment of the bands arising from the ν(SO_2) modes [21] The bands at (489), (462), (486) , (478), and (474) cm^{-1} were assigned to υ(M–O) for compounds (1), (2), (3), (4),and (5), indicating that to the carbocyclic oxygen, and oxygen of β-Lactam group of the ligand are involved in coordination with metal ions. The bands at (582), (513), (520), (551) and (540) cm^{-1} were assigned to υ(M–N) for compounds (1), (2), (3), (4), and (5), respectively , indicating that the amine nitrogen and nitrogen of (Sac) are involved in coordination with metal ions.

3.3 : The UV-Visible Spectroscopy and Magnetic measurements for the mixed ligand complexes

The values of band positions (λ_{max} nm)Cm^{-1} and molar absorptivity's (ε_{max} L cm^{-1} mol^{-1}) are listed in Table (5) together with the proposed assignments transition and suggested geometries . Magnetic moment values were calculated from the measured magnetic susceptibilities after employing diamagnetic corrections are listed in Table (6) . The (U.V- Vis) spectrum for the (amoxicillin) in DMSO solutions, exhibits a high intense absorption peak at (277 nm) (36101 cm-1) (ε_{max}=732 $molar^{-1}.cm^{-1}$), and a small peak at (330 nm) (30303 cm^{-1}) (ε_{max}=57 $molar^{-1}.cm^{-1}$), which assigned to (π→ π*), and (n→ π*) transition respectively [23] within the organic ligand,. The band in the 360-380 nm region is ascribed to an intraligand transition of the n→ π* type in accordance with the literature data for transitions due to sulphur atoms.
The results obtained are in good agreement with other spectra and the literature.[23 -26]

The magnetic moment (Table- 5)of the Ni(II) d^8 complex is 2.94 B.M Correspond to two unpaired electrons as expected for six coordinated spin free Ni (II) species. In the electronic spectrum of the Ni (II) complex shows three distinct bands appears at (282 nm) 35460 Cm^{-1}, (337 nm) 29673 $Cm^{-1}$and(380 nm) 26315 Cm^{-1} which may be assigned to Ligand .Feild , Charg .Transfer and (d-d) 3A2g(F) → 3T1g(P) (ν3)transition respectively are in good agreement with those for an octahedral geometry for nickel(II) complexes .

The Cu(II) d^9 spectrum, exhibit normal magnetic moments (1.75B.M.) which is in agreement with data reported by several research workers [19].This complex show broad asymmetric bands in the region at21413 Cm^{-1}, 29673 Cm^{-1} and 35460 Cm^{-1} assignable 2B1g → 2A1g, charge transfer and ligand field transitions Respectively[20]. These results reveal the distorted octahedral geometry for these complex. The former band may be due to 2Eg →2T2g accounted due to Jahn Teller effect suggesting thereby a distorted octahedral geometry for these complexes [21].
The (U.V- Vis) Co(II) d^9 spectrum, exhibits four peaks , the first high intense peak at (279 nm) (35842 cm^{-1})(ε max =2325 $molar^{-1}.cm^{-1}$), is due to the ligand field ,and (345 nm)(28985 cm^{-1}) (ε max =1901 $molar^{-1}. cm^{-1}$), is due to the charge transfer, and at 25445 and 14903 cm-1, there are assigned to 4T1 (F) →4T1 g) (υ2) 4T1g(F)→ 4A2g(F) (υ1) transition respectively, which are characteristic of octahedral stereo geometry : [22-24].,υ1/ υ2 = 0.59, υ2/ υ1 =1.70 from Tanabe-Sugano diagram for d7 octahedral field the value of Dq equal to 111.35 cm-1 , B'=950 cm^{-1},
The electronic spectra of d^{10}[Zn(II) ,C d(II)and Hg(II)]complexes do show the charge transfer , and the magnetic susceptibility shows that all complexes have diamagnetic moments., because d-d transitions are not possible hence electronic spectra did not give any fruitful information. in fact this result is a good agreement with

previous work of octahedral geometry. [21-25].proposed models of the species were built with chem. 3D shows in Figure(3).

4. Discussion of the biological effect results for prepared compounds:

The ligands and newly synthesized metal complexes were screened in vitro for their antibacterial activity against bacteria: ***Staphylococcus aureus*, *E-coli*** , ***Pseudomonas*** *and* ***Bacillus*** **.**

The zone of inhibition of the ligands and their complexes against the growth of bacteria were given in Table (5),Charts (1) were tested utilizing the agar diffusion technique [24].The organism tested were the agar media were inoculated with test organisms and a solution of the tested compound (100µg/ml) was placed separately in cups (10 mm diameter) in the agar medium. The plates were incubated for 24 h at (37°C) and the well was filled with the test solution using micropipette .During this period, the test solution was diffused and affected the growth of the inoculated microorganisms. Activity was determined by measuring the diameter of the zone showing complete inhibition (mm). Growth of inhibition was compared with the control (DMSO). The antibacterial activity results revealed that the ligands and there complexes shown weak to good activity when compared to the control. (DMSO).The increased inhibition activity of the metal complexes can be explained on the basis of Tweedy's chelation theory [26-27] . In metal complexes, on chelation the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, it increases the delocalization of π- electrons over the whole chelate ring. The ring of Saccharine moiety makes the complexes more lipophillic . [18-27]

Conclusion

In conclusion, the Mn (II),Fe(II),Co(II),Ni(II),Cu(II),Zn(II),Cd(II),and Hg (II)complexes of mixed Saccharin and Amoxicillin (antibiotics)are reported. The resultant complexes are characterized by melting point, conductivity measurement, UV-Vis and Infra-red spectroscopy. Investigation of antimicrobial activities was carried out against the tested organisms. All the complexes are found to be in octahedral geometry.

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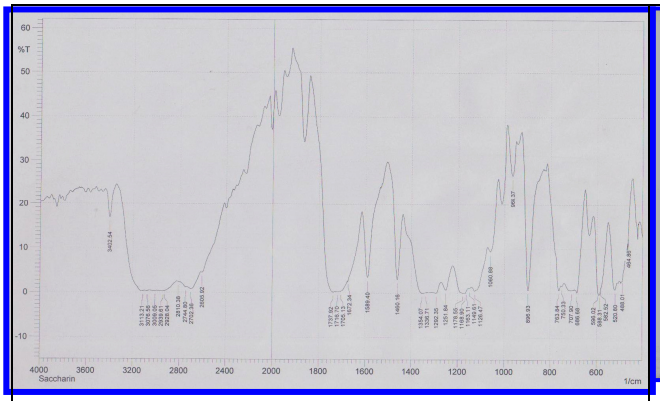
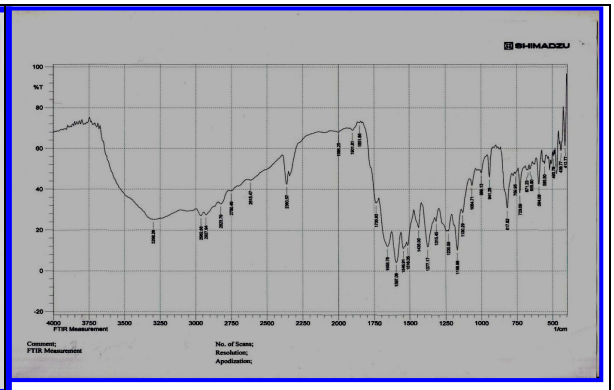
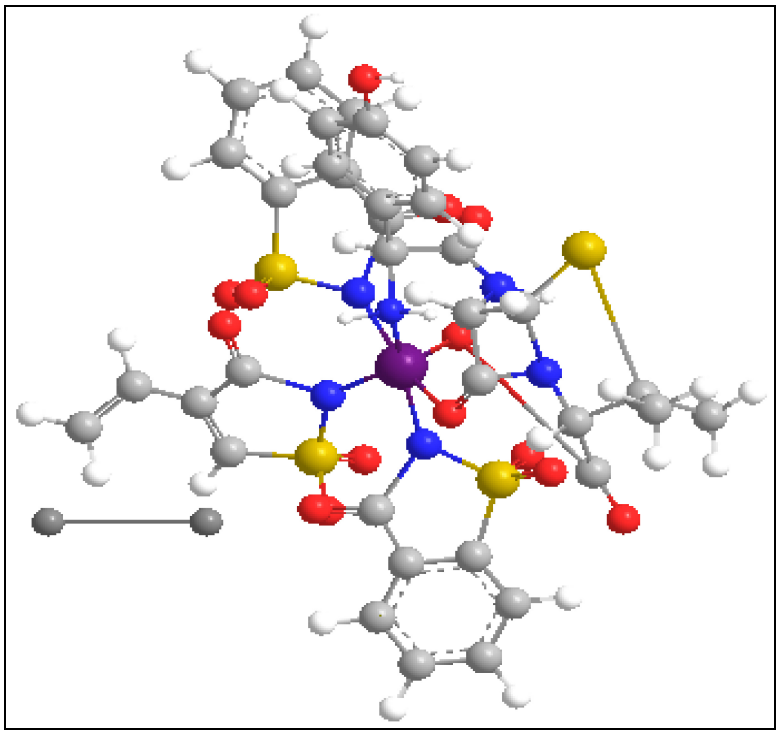


Figure (2): FT-IR spectrum of (Sac H)



Figure(1): FT-IR spectrum of (Amox)



***Figure(3)*3D molecular modeling proposed complexes**
Na_2[M (Amox)(Sac)$_3$]
Fe(II),Co(II),Ni(II),Cu(II), Zn(II),Cd(II)andHg(II)

Table (1)- The physical properties of the compounds

Compounds Chemical (Formula)	M. wt	Color	M .p °c (de) °c	Λ_m Ω^{-1} cm^2 mol^{-1} In DMSO	M%	Ml%
					Theory	experimental
Amox= $C_{16}H_{19}N_3O_5S$	365.10	White	205-196		-	-
SacH = $C_7H_5NO_3S$	182.18	Off White	113	12.4	-	-
Sac Na =$C_7H_4NO_3SNa$	205.166	Off Whit	-	-	-	-
$Na_2[Fe(Amox)(Sac)_3]$	1011.98	Grey green	236	57.43	5.51	4.97
$Na_2[Co(Amox)(Sac)_3]$	1014.98	Red-brown	115-118	62.45	5.80	5.13
$Na_2[Ni(Amox)(Sac)_3]$	1013.98	light Green	255Dec	68.23	5.78	5.27
$Na_2[Cu(Amox)(Sac)_3]$		Green	188	73.18	6.28	5.88
$Na_2[Zn(Amox)(Sac)_3]$	1019.97	Yellow	257Dec	66.42	6.40	6.07
$Na_2[Cd(Amox)(Sac)_3]$	1069.95	Off White	270Dec	54.76	10.51	9.93
$Na_2[Hg(Amox)(Sac)_3]$	1158.02	Yellow	214	60.15	17.33	16.85

Λm = Molar Conductivity, Dec. =decomposition ,

Table(2):Data from the Infrared Spectrum for the Free ligand Amoxicillin (cm^{-1})

Comp..	υ(O-H)	υ(N-H) primary amine	υ(N-H) Secondary amide	υ(C=O) for COOH	υ(C=O) β-lactam	υ_{asm} COO	υ_{asy} COO	υ(C=C) arom.	υ(C-C) aliph.	υ(C-N)	υ(C-O)	υ(C-S)	υ(C-H) arom.	υ(C-H) aliph.
Amox.	3525	3456	3176	1774	1685	1585	1396	1519	1178	1282	1249	557	3039	2970

Table(3):Data from the Infrared Spectrum for the Free Lingand Saccharin (cm^{-1})

	υ (N-H)asy υ (N-H)sym	υ (C - H) cy	υ_{asy} (SO_2)	υ_{ym} (SO_2)	(SO_2) υs/υa	υ(CNS)	υ(CO)	υ (CO)av
Sac H	3402s 3113br-vs	2928vs- sh	1292 vs	1149 vs	0.88	966s	1680 1705	1692

s: strong, m: medium, w: weak asy= asymmetric . sy= symmetric

Table(4)- Data from the Infrared Spectra for the \Metal Complexes (cm^{-1}).

	υ (O-H)	υ(N-H) primary amine	υ(N-H) Secondary amide	υ (C - H) cy &aliph	SO_2 v sy	υ(C=O) β-lactam	(SO_2) υ asy	υ_{asm} (COO-)	υ_{asy} (COO-)	Δ (COO-) asm - asm	υ (C=C) arom aliph.	υ (CNS)	υ(C-S)	M-O	M-N
1 Fe	3533	3344	3267	2962 2927	1292 vs	1647	1149 vs	1595 vs	1374	221	1516	972 vs	543	609w 532w	466 455
2 Co	3533	3387	3287	2981 2939	1284 vs	1643	1147 vs	vs 1585	1369	216	1516	968 m	540	609 578	451 408
3 Ni	br 3332	3332	3271	2981 2939	1288 vs	1612	1138 vs	Vs 1589	1369 vs	220	1516	952 s	543	601 509	474 424
4 Cu	3533	3444	3305	2958 2927	1284 vs	1741	1149 vs	1631 vs	1365 vs	266	1516	956s	540	601 505	439 412
5 Zn	3363 br. vs	vs3363	3363 br. vs	2981 2935	1284 vs	1728	1134 vs	1612 vs	1369 vs	243	1516	956 m	547	590 510	435 424
6 Cd	3533	3344	3287	2958 2927	1257 vs	1643	1145 vs	1589 vs	1369 vs	220	1512	960 m	538	570 609	482 451
7 Hg	3564 3483	3363	3066w	2978 2927	1280 vs	1639	1149 vs	1585	1369 s	216	1512	2s 67	543	605 528	482 424

Table (5) - Electronic Spectral data, magnetic moment of the mixed ligands complexes

Compounds	λ_{max}	ύ cm^{-1}	ε_{max} L. $mol^{-1}.cm^{-1}$	Assignments	Probable figure
Amox.	277 330	36101	732 57	π→ π* n→ π*	-
Sac					-
$Na_2[Fe(Amox)(Sac)_3]$	279 325 358 491	3542 30769 27932 20366	2297 1627 1208 216	L.F C-T C-T $^5T_2g \rightarrow {}^5Eg$	Distorted Octahedral
$Na_2[Co(Amox)(Sac)_3]$	279 345 393 671	35842 28985 25445 14903	2325 1901 1073 527	L.F C-T $^4T1\ (F) \rightarrow {}^4T_1\ g\ )$ $^4T1(F) \rightarrow {}^4T_2g(F)$	Octahedral
$Na_2[Ni(Amox)(Sac)_3]$	282 337 380	35460 29673 26315	2410 1783 1077	L.F C.T $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ (ν_3)	Octahedral
$Na_2[Cu(Amox)(Sac)_3]$	280 370 467	35714 27027 21413	2339 1642 374	L.F C.T $^2E_g \rightarrow {}^2T_{2g}$	distorted Octahedral
$Na_2[Zn(Amox)(Sac)_3]$	274 361	36496 27700	2033 715	C.T C.T	Octahedral
$Na_2[Cd(Amox)(Sac)_3]$	285 363	35087 27548	2451 1135	C.T C.T	Octahedral
$Na_2[Hg(Amox)(Sac)_3]$	279 345 372	35842 28985 26881	2385 2142 1542	C.T C.T	Octahedral

Table (6) - Data magnetic moment of the mixed ligands Complexes

Complex	$X_g.10^{-6}$	$X_m.10^{-6}$	$X_A.10^{-6}$	μ_{eff} (B.M)
$Na_2[Fe(Amox)(Sec)_3]$	1.00	979.326	1174.496	5.23
$Na_2[Co(Amox)(Sec)_3]$	6.123	6215.296	6410.466	3.87
$Na_2[Ni(Amox)(Sec)_3]$	3.456	3504.494	3699.664	2.94
$Na_2[Cu(Amox)(Sec)_3]$	1.095	1115.652	1310.822	1.75

Table(7)- Biological activity of the mixed ligands complexes (Zone of inhibition) (mm)

Compound	*E-coli*	*Pseudomonas*	*Staphylococcus aureus (+ve)*	*Bacillus*
Control(DMSO)	5	7	5	5
Amox.	0	16	0	8
SacH	6	5	9	6
$Na_2[Fe(Amox)(Sac)_3]$	0	0	0	0
$Na_2[Co(Amox)(Sac)_3]$	0	0	0	0
$Na_2[Ni(Amox)(Sac)_3]$	0	0	0	0
$Na_2[Cu(Amox)(Sac)_3]$	0	0	0	0
$Na_2[Zn(Amox)(Sac)_3]$	0	0	0	0
$Na_2[Cd(Amox)(Sac)_3]$	16	14	16	23
$Na_2[Hg(Amox)(Sac)_3]$	14	14	12	0

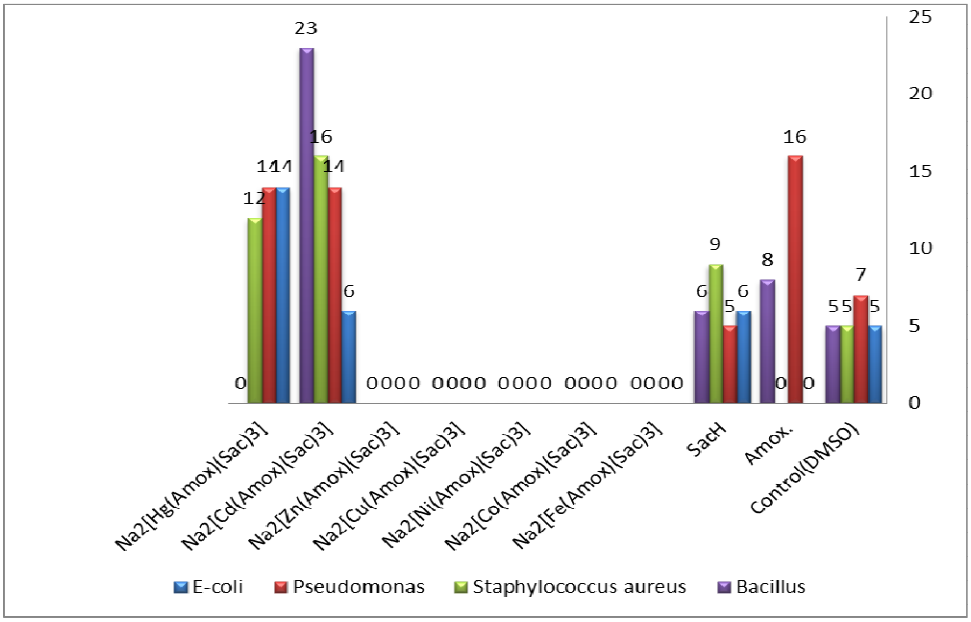


Chart (1) Chart of biological effects of the studied complexes